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Peripheral Fat Loss and Decline in Adipogenesis in Older Humans

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Abstract

Objective—Aging is associated with a redistribution of body fat including a relative loss of subcutaneous peripheral fat. These changes in body fat can have important clinical consequences since they are linked to increased risk of metabolic complications. The causes and mechanisms of loss of peripheral fat associated with aging are not clear. The aim of this study was to assess whether defects in adipogenesis contribute to fat loss in aging humans, as suggested from animal studies, and to evaluate the role of inflammation on pathogenesis of fat loss.

Materials/Methods—Preadipocytes isolated from subcutaneous peripheral fat of healthy young and elderly subjects were compared in their ability to replicate and differentiate.

Results—The results show that both the rate of replication and differentiation of preadipocytes are reduced in older subjects. The reduction in adipogenesis is accompanied by a higher plasma level of the inflammatory marker, soluble tumor necrosis factor receptor 2, and greater release of tumor necrosis factor α from fat tissue.

Conclusions—Thus, the gradual relative loss of peripheral fat in aging humans may in part result from a defect in adipogenesis, which may be linked to inflammation and increased release of proinflammatory cytokines from fat tissue.

Keywords

Adipogenesis; aging; inflammation; preadipocytes; tumor necrosis factor α

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AUTHOR CONTRIBUTION

G.C., M.A.M., and M.C.G. contributed to the project conception, study design and overall research plan; D.C.M. participated in discussions of concept and design of studies; A.Z. collected the fat samples; G.C. and I.M. conducted the experiments and sample analysis; G.C. and M.A.M. analyzed data and wrote the manuscript.

CONFLICT OF INTEREST

None of authors has any conflict of interest

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INTRODUCTION

Aging is associated with redistribution of body adipose tissue with a relative loss of subcutaneous adipose tissue particularly from the limbs and accumulation of adipose tissue in trunk and visceral areas (e.g.[1, 2]). This loss of peripheral subcutaneous fat is associated with metabolic abnormalities, particularly insulin resistance [3, 4] with increased risk of diabetes [5] and cardiovascular disease [6].

The mechanism of subcutaneous peripheral fat loss with aging is not completely understood. Observations in rodents suggest that this decline in peripheral body fat is associated with a decrease in the ability of preadipocytes to differentiate [7, 8]. Since new fat cells are continually produced from preadipocytes [9], age-related alterations in the number of preadipocytes may also contribute to the loss of fat. Aging is accompanied by chronic inflammation, which has been linked to insulin resistance and other age-related pathologies [3, 10, 11]. There is *in vitro* evidence that inflammatory cytokines, particularly tumor necrosis factor α (TNF α), exert a suppressive effect on differentiation of preadipocytes [12, 13]. However, currently, little is known about the role of inflammation in the loss of peripheral adipose tissue in aging humans.

The aim of this study was to assess preadipocyte replication and differentiation in older adults, in the context of loss of peripheral adipose tissue. The role of inflammation in the loss of peripheral adipose was assessed from circulating levels of soluble tumor necrosis factor receptor 2 (sTNFR2) and the release of TNF α from fat tissue. A better understanding of the mechanism of loss of peripheral adipose tissue in aging provides an important first step in the identification of potential therapeutic targets for the elderly population.

MATERIAL AND METHODS

Study subjects

Twenty-seven healthy subjects (15 young, age 18–30 y, mean 27 ± 1 y; and 12 elderly, age > 60 , mean 71 ± 2 y), screened to exclude abnormality of liver or kidney function, diabetes, hematologic and endocrine diseases, acute inflammatory diseases or illnesses, participated in the study. Fat distribution was assessed by dual X-ray absorptiometry. Subjects provided a fasting blood sample and underwent a surgical procedure under local anesthesia for removal of a subcutaneous fat sample from the outer mid-thigh. Specimens were immediately placed in sterile Hank's buffered salt solution containing antibiotics. The protocol was approved by the Stony Brook University Institutional Review Board and all subjects gave written informed consent.

Subcutaneous peripheral (i.e. leg) pre-adipocyte isolation and culture

Preadipocytes were immediately isolated under aseptic conditions with collagenase digestion and removal of contaminating red blood cells [14]. Stromal cells were plated in DMEM/F-12 (Gibco, Carlsbad, CA) supplemented with 10 % fetal calf serum. After 16–18 h, attached cells were extensively washed with warm phosphate-buffered saline, detached and counted. Preadipocytes were then plated a density of $5 \times 10^3/\text{cm}^2$ for growth and differentiation experiments.

Assessment of preadipocyte proliferation

Preadipocyte number was assessed over 168 h with a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide based assay [14, 15]. Proliferation rate of preadipocytes was assessed by calculating the slope of the growth curve (i.e. cell number vs. time).

Assessment of preadipocyte differentiation

Cells were grown until confluent. Differentiation was induced with serum-free medium containing 0.5 $\mu\text{mol/L}$ insulin, 0.1 $\mu\text{mol/L}$ dexamethasone, 0.5 nmol/L triiodothyronine, 0.5 $\mu\text{mol/L}$ rosiglitazone, 540 $\mu\text{mol/L}$ 3-isobutyl-1-methylxanthine [14]. Preadipocyte differentiation was quantified at 12 d from the intracellular lipid accumulation after oil red O staining, following extraction and spectrophotometric quantification [14]. The percentage of differentiated preadipocytes was also estimated by directly counting cultures stained with oil red O and counterstained with hematoxylin to visualize nuclei/cell contours, as we previously described [14].

TNF- α release by adipose tissue

After collection, a portion of fat tissue was minced, resuspended in Krebs-Ringer lactate buffer containing 4% albumin (pH 7.0), and incubated at 37 °C for 4 h. Following centrifugation and storage at -70°C, supernatants were assayed for TNF- α levels by ELISA (R&D Systems, Minneapolis, MN).

Plasma sTNFR2 concentration

Plasma levels of sTNFR2 were measured by ELISA (Invitrogen, Carlsbad, CA).

Statistics

Results are expressed as means $\pm$ SEM. Differences between groups were analyzed by unpaired Student's t-tests with P values < 0.05 considered statistically significant. Linear regression models were used to study the association between age and multiple variables including analyses controlling for differences in body fat. Normal distribution was confirmed and the analysis was performed using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

Elderly subjects had a mean age of 71 ± 2 y and young subjects had a mean age of 27 ± 1 y. None of the enrolled subjects was taking any medication, with the exception of standard multivitamin supplements. Compared to young, elderly subjects had a higher body mass index (BMI, 26.2 ± 0.7 vs. 23.6 ± 0.7 kg/m², $P = 0.02$) and an overall greater amount of total body (9.06 ± 0.63 vs. 5.70 ± 0.65 kg/m², $P < 0.002$), trunk (4.32 ± 0.34 vs. 2.19 ± 0.31 kg/m², $P < 0.001$) and limb fat (4.39 ± 0.44 vs. 3.14 ± 0.37 kg/m², $P < 0.05$) normalized for height. Older subjects had a smaller proportion of body fat located in the limbs and higher fat accumulation in the abdominal area, as indicated by the significantly lower limb fat to trunk fat ratio in the elderly group (1.09 ± 0.13 vs. 1.50 ± 0.10 , $P < 0.05$).

Preadipocytes were isolated from biopsies of adipose tissue taken from the outer aspect of the thigh and then assessed *in vitro* for the rate of proliferation and differentiation.

Preadipocyte proliferation

The growth rate of preadipocytes was significantly slower in elderly than in young subjects (Figure 1: slope: $1.31 \pm 0.09 \times 10^{-3}$ vs. $1.71 \pm 0.09 \times 10^{-3}$, $P = 0.005$). The rate of preadipocyte proliferation is inversely correlated with BMI ($R=0.44$, $P=0.026$), total body fat ($R=0.64$, $P<0.001$), trunk fat ($R=0.59$, $P=0.003$) and total limb fat ($R=0.58$, $P=0.004$) normalized for height (i.e. g/m²). However, linear regression analysis controlling for body fat indicates that there is a significant difference in proliferation associated with age which is independent of body fat ($P = 0.04$).

Preadipocyte differentiation

Preadipocyte differentiation assessed by quantification of intracellular lipid accumulation was significantly reduced by 25% in elderly subjects compared to young subjects (absorbance 0.214 ± 0.016 vs. 0.286 ± 0.026 , $P < 0.05$) (Figure 3, supplemental appendix). When differentiation was assessed as the proportion of cells which show some lipid accumulation, the number of differentiated preadipocytes also tended to be lower (-14%) in the elderly than in the young group (59.5 ± 3.9 vs. $69.2\% \pm 3.7$, $P = 0.07$). Unlike preadipocyte proliferation, preadipocyte differentiation is not correlated with any of the measures of body fat (i.e. BMI, total body fat/m², trunk fat/m², or limb fat/m²). However, preadipocyte differentiation is positively related to body fat distribution, i.e. body fat present in the limbs relative to body fat present in the trunk (limb fat g/trunk fat g) whether differentiation is expressed as lipid accumulation ($R=0.59$, $P=0.003$, Figure 2) or proportion of differentiated cells ($R=0.48$, $P=0.02$).

The plasma levels of the inflammatory marker sTNFR2 was 30% higher in the elderly compared to young subjects (3.40 ± 0.27 ng/mL vs 2.57 ± 0.21 , $P = 0.02$). TNF- α release from fat tissue was also significantly elevated in the elderly group (4.60 ± 1.16 pg/mg tissue vs 0.97 ± 0.22 , $P = 0.007$). A significant inverse relationship is observed between preadipocyte replication and sTNFR2 plasma levels ($R = 0.35$, $P=0.03$) and TNF- α release from adipose tissue ($R = 0.53$, $P=0.04$). An inverse correlation is also observed between preadipocyte differentiation and sTNFR2 plasma levels ($R=0.35$, $P= 0.09$) and TNF α release from adipose tissue ($R=0.4$, $P=0.1$), which approach statistical significance. Concentrations of sTNFR2 in plasma are also positively related to total body fat normalized for height ($R=0.46$, $P=0.02$) and TNF- α release from adipose tissue is positively related to total body fat ($R=0.70$, $P<0.001$), trunk fat ($R=0.58$, $P=0.03$) and limb fat ($R=0.6$, $P=0.024$) normalized for height. Linear regression modeling of the relationship of age to sTNFR2 and TNF- α release indicates that even after adjusting for body fat, there is a significant relationship between age and sTNFR2 in plasma ($P=0.05$) and release of TNF α from adipose tissue ($P=0.03$).

DISCUSSION

This study demonstrates, for the first time, that human aging is associated with changes in preadipocytes from subcutaneous adipose tissue which affect both the size of the preadipocyte pool available for differentiation and the rate at which these cells progress to form fully differentiated adipocytes. These changes are associated with a relative decline in the proportion of adipose tissue in the periphery which has serious implications for insulin resistance [3,5] and heart disease [6]. Moreover, these changes in preadipocyte replication and differentiation in older humans are associated with an elevation in the pro-inflammatory cytokine, TNF α , which suggests the possible benefit of anti-inflammatory therapy in preventing the age-related alteration in peripheral adipose tissue.

A highly significant positive relationship between the capacity of preadipocytes to differentiate and the distribution of adipose tissue between the periphery and the trunk is demonstrated both when adipose tissue distribution is expressed as a ratio of limb fat to trunk ($R = 0.59$, $P = 0.003$, Fig. 2) and when adipose tissue distribution is expressed as the proportion of total fat present in the limbs ($R = 0.55$, $P = 0.008$). Thus, this relative loss of adipose tissue in the periphery is associated with both a reduction in the number of precursor cells available for differentiation due to reduced capacity of preadipocytes to proliferate and to a reduction in the capacity of preadipocytes to differentiate.

An age-related reduction in capacity for preadipocyte replication, differentiation, and lipid accumulation is consistent with data from old rats compared to young animals [8, 16]. In

rodents, the mechanism for decreased differentiation of preadipocytes is linked to a reduced expression of key transcription factors such as CCAAT/enhancer binding protein linked to TNF α [7, 17]. In elderly subjects, impairment of adipogenesis was also accompanied by a significant elevation in the plasma concentration of the inflammatory marker, sTNFR2, and also by significantly enhanced release of TNF α by adipose tissue in primary culture. The greater amount of TNF α released from subcutaneous peripheral fat of older subjects suggests that a proinflammatory state in adipose tissue with aging can contribute to the gradual loss of peripheral fat. In addition to depressing differentiation and maturation of preadipocytes [18, 19], TNF α can also promote fat loss by counteracting insulin action and inducing insulin resistance in fat cells and stimulating lipolysis [18, 19]. We have previously shown that insulin resistance is associated with inflammation including elevated plasma sTNFR2 and a reduction in limb fat relative to trunk fat [3, 4].

In summary, this study shows that the loss of peripheral fat associated with aging may, in part, result from a decline in adipogenesis. Both the rate of replication of subcutaneous peripheral preadipocytes and their ability to differentiate are impaired with aging. The present study also suggests that the defect in adipogenesis may be linked to inflammation and in particular to a proinflammatory state of the subcutaneous adipose tissue, characterized by an increased release of TNF α and possibly other cytokines having an inhibitory paracrine action on fat cell metabolism and differentiation. The role of inflammation on fat loss and the efficacy of therapeutic strategies targeted at reducing systemic and tissue inflammation may be important in the prevention of fat loss and reduction of metabolic complication associated with aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

sTNFR2	tumor necrosis factor receptor 2
TNF α	tumor necrosis factor α

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Figure 1. Proliferation of preadipocytes isolated from subcutaneous fat of Young and Elderly subjects

Cell number was assessed with the MTT assay at 48, 72, 96, 120, 144 and 168 h (Mean $\pm$ SEM). The slope of the growth curve was significantly lower in Elderly Subjects ($P < 0.01$).

Figure 2. Relationship between subcutaneous preadipocyte differentiation and ratio of limb to trunk fat (kg/kg)

Differentiation was assessed with Oil Red O staining of intracellular lipid. A comparable relationship was obtained when differentiation was assessed by direct count of the proportion of differentiated cells ($R = 0.48$, $P = 0.02$).

○ young; □ elderly

Figure 3. (Supplemental online appendix). Differentiation of preadipocytes isolated from subcutaneous fat of Young and Elderly subjects

Preadipocytes were differentiated for 12 d and then intracellular lipid were stained with Oil Red O. a. Pictures (40X) of stained adipocytes from young and elderly subjects showing intracellular lipid accumulation in red. b. Spectrophotometric quantification of Oil Red O extracted from differentiated preadipocytes in the 2 groups. Mean $\pm$ SEM. * $P < 0.05$